Perspective

Progress With Nonhuman Animal Models of Addiction

ABSTRACT. Nonhuman animals have been major contributors to the science of the genetics of addiction. Given the explosion of interest in genetics, it is fair to ask, are we making reasonable progress toward our goals with animal models? I will argue that our goals are changing and that overall progress has been steady and seems likely to continue apace. Genetics tools have developed almost incredibly rapidly, enabling both more reductionist and more synthetic or integrative approaches. I believe that these approaches to making progress have been unbalanced in biomedical science, favoring reductionism, particularly in animal genetics. I argue that substantial, novel progress is also likely to come in the other direction, toward synthesis and abstraction. Another area in which future progress with genetic animal models seems poised to contribute more is the reconciliation of human and animal phenotypes, or consilience. The inherent power of the genetic animal models could be more profitably exploited. In the end, animal research has continued to provide novel insights about how genes influence individual differences in addiction risk and consequences. The rules of the genetics game are changing so fast that it is hard to remember how comparatively little we knew even a generation ago. Rather than worry about whether we have been wasting time and resources asking the questions we have been, we should look to the future and see if we can come up with some new ones. The valuable findings from the past will endure, and the sidetracks will be forgotten. (*J. Stud. Alcohol Drugs, 77,* 696–699, 2016)

THAT GENETICS, BROADLY CONSTRUED, IS among the most rapidly changing areas of the biological sciences seems incontestable. Studies attempting to understand the relationships between the collection of dysfunctional and/or self-destructive behaviors engaged in by a drug-addicted human and the particularities of his or her genome encompass the science of the genetics of addiction. Given the explosion of interest in genetics, it is fair to ask, are we making reasonable progress toward our goal? It could easily seem that we are expending too many resources in this area to achieve but modest gains, and I have been charged with considering this specifically regarding nonhuman animal studies. The initial goal was, broadly, to identify the genes of importance, then identify their gene products and roles in the brain to enable better understanding of pathophysiology and aid in identifying novel pharmacotherapeutics. I will argue that our goals are changing and that, overall, progress has been steady and seems likely to continue apace.

Animal studies first indicated the importance of genetics to alcohol-related behavior in the late 1940s and early 1950s. By the end of the 1970s, there was already a substantial literature based on several naturally occurring or intentionally created genetic variants of rats and mice. Principally, these were different sets of essential clones (inbred strains) and rodents purposefully bred to drink more or less alcohol by choice (selectively bred lines) (Lumeng et al., 1977; Mardones & Segovia-Riquelme, 1983; McClearn & Rodgers, 1959). We used these models to explore the extent of genetic influences on a range of behaviors and neurobiological correlates comprising alcohol's pharmacology, and that compilation has been expanding rapidly ever since. As the human genetics literature progressed from being able to assert the heritability of an addiction diagnosis to detecting the measurable influence of one versus another allele at a handful of candidate genes, or the presence of statistically verifiable covariance of addiction with other traits, the animal literature in parallel could identify naturally occurring gene variations affecting drug sensitivity and began to develop the capability of studying expression, the other half of the genetic pathway.

Genes, of course, are not generally tonically transcribed to make their functional proteins, and the ability to take apart an animal's DNA, or brain, or liver, has always been much more frequently available for nonhuman studies. In early animal studies, however, no single gene seemed to produce a major, Mendelian effect on addiction traits as important as the discovery of the ALDH2*2 enzyme variant in humans. This polymorphism confers slow clearance of acetaldehyde, a toxic metabolite of alcohol, to many East Asian individuals and reduces the risk of an alcoholism diagnosis substantially. Disulfiram (Antabuse) mimics this enzyme deficiency and was the first drug treatment approved by the U.S. Food and Drug administration to treat alcoholism (Enoch & Goldman, 2001).

The last 25 years have seen an explosion of scientific findings on all fronts. In both animal and human genetics, the tools have developed almost incredibly rapidly in the directions of enabling both more reductionist and more synthetic or integrative approaches. I believe that these two

approaches to making progress are always unbalanced in biomedical science, favoring reductionism, particularly in animal genetics. For example, first we sought to pinpoint in the genome the chromosomal location of specific genes influencing addiction traits (Crabbe et al., 1994). Proving a gene's influence was greatly facilitated by being able to produce transgenic mice with the gene's transcription disrupted or enhanced (e.g., Rubinstein et al., 1997), and then by being able to do so conditionally (e.g., in specific brain areas, or during specific developmental periods; see Nelson & Young, 1998). Different ways of studying DNA moved us to a more differentiated level of description of a gene, using actual base pairs rather than genetic markers. Sequencing DNA became routine as a less expensive way to identify errors (Hitzemann et al., 2013). We know now that genes often encode multiple RNAs differing in length (splice variants), which may direct synthesis of different proteins or no protein at all (Iancu et al., 2014; Tavares et al., 2015).

In parallel, we learned from early, gene-by-gene studies that linking an addiction trait to an individual's genome requires a deep understanding of gene expression. Sequencing RNA has already begun to replace the use of microarray chips to characterize gene expression patterns. We have learned that thousands of RNAs that do not code for genes instead participate intimately in the regulation of gene transcription (Barbierato et al., 2015).

Human genetic studies have participated in this explosion of knowledge regarding gene sequence differences, and the human and nonhuman genomic data have been developed in parallel. However, nonhuman animal studies have always been able to reveal more faster about potential underlying mechanisms, which is their great contribution. This is, of course, generally true about biological processes and associated interventions and is not limited to genetic studies. We can explore the brains of rodents directly, comparing brain regions, cell types, circuits, and pathways, and aligning the functional consequences of gene sequence and expression manipulations with specific genes.

Very recently, the CRISPR/Cas9 gene editing technique has allowed us to perform a gene therapy experiment easily and with precision, for example, by replacing a functional gene with a dysfunctional variant without waiting for two generations of rodent breeding to learn the result (Shalem et al., 2015). The other major advantage with nonhuman studies is that we control who breeds with whom. The oldest technique in genetics involves choosing breeding partners to study inheritance patterns. We have built many genetic animal models for traits related to addiction, mostly to alcohol, but also to other drugs of abuse. We have also bred animals to display traits we believe to represent different addiction risks or symptoms (Noronha et al., 2014). Animal studies have recently shown us that Jean-Baptiste Lamarck (1744–1829) was not entirely incorrect and that certain so-called epigenetic biochemical modifications of the genome caused by experience can be transmitted in the germ line to future generations (Isles, 2015). This has laid the final nails in the coffin of Genes versus Environment as mutually exclusive sources of generational influence—genes and environment interact in many ways and at all levels of analysis.

I would argue that substantial and novel progress is likely to come in the other direction as well, toward synthesis and abstraction. This is supported by the data derived from all the genomic information accumulated to this point, plus the rapid increase in development of informatics tools to exploit them. From both human and nonhuman studies, we are finding inescapably that seeking to trace genetic influence to individual genes (be it in sequence or expression) tends to lead us to find a large number of genes, each with a very small influence on our studied trait. In the aggregate, we find that a substantial proportion of individual difference variation can be ascribed to a collection of many genes and their interactive effects on each other and on the trait.

Although some of those genes may prove to have a very marked influence in a restricted population of individuals or families, knowing the whole list of an individual's "risk" genes is not likely to help explain the biological source of his or her symptoms, or, therefore, direct us to a specific or novel therapy. In the animal literature, the newest gene-finding efforts are now focusing on applying powerful multivariate statistical analyses to identify the influence of clusters of gene variation. One example is the use of the same sort of network analysis that Facebook uses to survey your network of friends, and their friends, and which of you from that gigantic set are actively contacting one another on Friday nights seeking to meet to shop for X: Facebook's goal is to direct advertising your way. You will receive this advertising based on your associates' histories, whether or not you happen to be interested in a mall that particular Friday. In animal studies, patterns of correlated gene expression are in this way mined to try to identify potential "hub" genes that importantly seem to predict (and perhaps directly regulate) the expression of many other genes (Iancu et al., 2013).

Genomics and informatics are both omnivorous and catholic about data. Informatics practitioners are willing to explore multiple databases and relate them to other databases. For example, very creative ways are being developed for mining the scientific literature using keyword and text searches and relating written content to genomic and biological data (Baker et al., 2012). I am no expert in this field, but there is a parallel with the increased use by biomedical science of meta-analyses to assess the range of clinical outcomes from clinical trial data. Meta-analysis has developed very clear and stringent rules about which trials to allow into such a study and which to exclude. My sense is that synthetic genetic and informatic studies are still a bit in the domain of searching for good compelling stories (Bubier et

al., 2014; Zhou et al., 2013) but that we have yet to accumulate enough experience with these stories after they are proposed from initial studies to understand the range of their ultimate utility.

Another area in which future progress with genetic animal models seems poised to contribute more is the reconciliation of human and animal phenotypes, or consilience. Human behavior can be pretty accurately modeled in the laboratory because we get a great deal of help from subjects with the interpretation of each laboratory assay. Animals are unable to guide us, and, particularly at the behavioral level, we must infer what their behavior "means." We have spent far too long using and reusing the same, very restricted set of rodent behavioral assays and are far too fond of labeling the resulting behavioral output with a human construct (anxiety, depression, hyperactivity, impulsivity, loss of control, compulsivity, etc). The inherent power of the genetic animal models could be more creatively used to advance the exploration of the wealth of reductionistically derived data.

The new gene editing methods (e.g., CRISPR/Cas9) have already been used to manipulate multiple genes at once (Shalem et al., 2015; Walters et al., 2015), and such use seems likely to spread. Propositions about the role of specific genetically modulated pathways could then be fairly powerfully tested. This suggests to me that our goals regarding the "genetics" of addiction are also changing. The tools of genetic manipulation have become so powerful that genetic advances are rapidly reshaping the neurobiological questions we are asking. Using sophisticated molecular techniques—such as optogenetics and Daun02 inactivation—we can selectively target specific cell types and track their functions in neural ensembles, giving us novel ideas about brain plasticity (Cruz et al., 2013; Ferenczi & Deisseroth, 2016).

So, in the end, research with nonhuman animals has continued to provide novel insights about how genes influence individual differences in addiction risk and consequences. The rules of the genetics game are changing so fast that it is difficult to remember how comparatively little we knew even a generation ago. Progress in human genetics has benefited from the same reductionist methodological onslaught, which constantly changes the questions. Rather than worry about whether we have been wasting time and resources asking the questions we have been asking, we should look to the future and see if we can produce some new ones. The valuable findings from the past will endure, and the sidetracks will be forgotten.

John C. Crabbe, ph.d.[a]
crabbe@ohsu.edu

[a]*Department of Behavioral Neuroscience, Oregon Health & Science University, Veterans Affairs Portland Health Care System Portland, Oregon*

Acknowledgment

Support for the writing of this article was provided by U.S. Department of Veterans Affairs Grant 101BX000313 and by National Institutes of Health Grants AA013519, AA010760, AA020245, and DA018165.

Conflict of Interest Statement

The author has no conflicts of interest to report.

References

Baker, E. J., Jay, J. J., Bubier, J. A., Langston, M. A., & Chesler, E. J. (2012). GeneWeaver: A web-based system for integrative functional genomics. *Nucleic Acids Research, 40, Database issue,* D1067–D1076. doi:10.1093/nar/gkr968

Barbierato, M., Zusso, M., Skaper, S. D., & Giusti, P. (2015). MicroRNAs: Emerging role in the endogenous μ opioid system. *CNS & Neurological Disorders - Drug Targets, 14,* 239–250. doi:10.2174/1871527314666150116123932

Bubier, J. A., Jay, J. J., Baker, C. L., Bergeson, S. E., Ohno, H., Metten, P., . . . Chesler, E. J. (2014). Identification of a QTL in *Mus musculus* for alcohol preference, withdrawal, and *Ap3m2* expression using integrative functional genomics and precision genetics. *Genetics, 197,* 1377–1393. doi:10.1534/genetics.114.166165

Crabbe, J. C., Belknap, J. K., & Buck, K. J. (1994). Genetic animal models of alcohol and drug abuse. *Science, 264,* 1715–1723. doi:10.1126/science.8209252

Cruz, F. C., Koya, E., Guez-Barber, D. H., Bossert, J. M., Lupica, C. R., Shaham, Y., & Hope, B. T. (2013). New technologies for examining the role of neuronal ensembles in drug addiction and fear. *Nature Reviews Neuroscience, 14,* 743–754. doi:10.1038/nrn3597

Enoch, M. A., & Goldman, D. (2001). The genetics of alcoholism and alcohol abuse. *Current Psychiatry Reports, 3,* 144–151. doi:10.1007/s11920-001-0012-3

Ferenczi, E., & Deisseroth, K. (2016). Illuminating next-generation brain therapies. *Nature Neuroscience, 19,* 414–416. doi:10.1038/nn.4232

Hitzemann, R., Bottomly, D., Darakjian, P., Walter, N., Iancu, O., Searles, R., . . . McWeeney, S. (2013). Genes, behavior and next-generation RNA sequencing. *Genes, Brain, & Behavior, 12,* 1–12. doi:10.1111/gbb.12007

Iancu, O. D., Colville, A., Darakjian, P., & Hitzemann, R. (2014). Coexpression and cosplicing network approaches for the study of mammalian brain transcriptomes. *International Review of Neurobiology, 116,* 73–93. doi:10.1016/B978-0-12-801105-8.00004-7

Iancu, O. D., Oberbeck, D., Darakjian, P., Metten, P., McWeeney, S., Crabbe, J. C., & Hitzemann, R. (2013). Selection for drinking in the dark alters brain gene coexpression networks. *Alcoholism: Clinical and Experimental Research, 37,* 1295–1303. doi:10.1111/acer.12100

Isles, A. R. (2015). Neural and behavioral epigenetics; what it is, and what is hype. *Genes, Brain, & Behavior, 14,* 64–72. doi:10.1111/gbb.12184

Lumeng, L., Hawkins, T. D., & Li, T.-K. (1977). New strains of rats with alcohol preference and non-preference. In R. G. Thurman, J. R. Williamson, H. Drott, & B. Chance (Eds.), *Alcohol and aldehyde metabolizing systems* (pp. 537–544). New York, NY: Academic Press.

Mardones, J., & Segovia-Riquelme, N. (1983). Thirty-two years of selection of rats by ethanol preference: UChA and UChB strains. *Neurobehavioral Toxicology and Teratology, 5,* 171–178.

McClearn, G. E., & Rodgers, D. A. (1959). Differences in alcohol preference among inbred strains of mice. *Quarterly Journal of Studies on Alcohol, 20,* 691–695.

Nelson, R. J., & Young, K. A. (1998). Behavior in mice with targeted disruption of single genes. *Neuroscience and Biobehavioral Reviews, 22,* 453–462. doi:10.1016/S0149-7634(97)00053-5

Noronha, A. B. C., Cui, C., Harris, R. A., & Crabbe, J. C. (2014).

Neurobiology of alcohol dependence. San Diego, CA: Elsevier/Academic Press.

Rubinstein, M., Phillips, T. J., Bunzow, J. R., Falzone, T. L., Dziewczapolski, G., Zhang, G., . . . Grandy, D. K. (1997). Mice lacking dopamine D4 receptors are supersensitive to ethanol, cocaine, and methamphetamine. *Cell, 90,* 991–1001. doi:10.1016/S0092-8674(00)80365-7

Shalem, O., Sanjana, N. E., & Zhang, F. (2015). High-throughput functional genomics using CRISPR-Cas9. *Nature Reviews Genetics, 16,* 299–311. doi:10.1038/nrg3899

Tavares, R., Scherer, N. M., Ferreira, C. G., Costa, F. F., & Passetti, F. (2015). Splice variants in the proteome: A promising and challenging field to targeted drug discovery. *Drug Discovery Today, 20,* 353–360. doi:10.1016/j.drudis.2014.11.002

Walters, B. J., Azam, A. B., Gillon, C. J., Josselyn, S. A., & Zovkic, I. B. (2015) Advanced in vivo use of CRISPR/Cas9 and anti-sense DNA inhibition for gene manipulation in the brain. *Frontiers in Genetics, 6,* 362. doi:10.3389/fgene.2015.00362

Zhou, Z., Karlsson, C., Liang, T., Xiong, W., Kimura, M., Tapocik, J. D., . . . Goldman, D. (2013). Loss of metabotropic glutamate receptor 2 escalates alcohol consumption. *Proceedings of the National Academy of Sciences of the United States of America, 110,* 16963–16968. doi:10.1073/pnas.1309839110